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New synthesis of all four 1-amino-2-hydroxycyclohexanecarboxylic acids

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Abstract—This report describes a new synthesis of the four stereoisomers of 1-amino-2-hydroxycyclohexanecarboxylic acids [(1*S*,2*S*)-, (1*R*,2*R*)-, (1*S*,2*R*)- and (1*R*,2*S*)-c_6Ser], four conformationally constrained serine (Ser) analogues, possessing a six-membered carbocyclic ring. Initially, we synthesised *cis*-c_6Ser and *trans*-c_6Ser in their racemic forms, using as key steps the Diels–Alder reactions of methyl 2-benzamidoacrylate with Danishefsky's diene and 1-methoxy-1,3-butadiene, respectively. The optically active forms were achieved by resolution methods.

1. Introduction

Due to the important role that L-serine (L-Ser) plays in peptides,[1–5] not only as an hydrophilic residue but also as either an active site or a catalytic site of a variety of enzymatic transformations, in recent years there has been an increasing interest in the synthesis of conformational variants of L-Ser, with the aim of incorporating them into peptides instead of L-Ser.

In this context, 1-amino-2-hydroxycyclohexanecarboxylic acid (c_6Ser) has received considerable attention since high rigidity is achieved in this molecule by having the α-carbon of the amino acid incorporated into a six-membered ring. The first approach to this target molecule was carried out by Christensen and co-workers,[6] obtaining the serine analogues *cis*- and *trans*-c_6Ser as a mixture of stereoisomers and in a racemic form via the Bucherer–Libe and Strecker reactions from 2-hydroxycyclohexanone. These amino acids were used to study the cellular entry of α-amino acids through the plasma membrane of the Ehrlich cell.[6]

Later, Ohfune and co-workers,[7,8] using an intramolecular version of the asymmetric Strecker reaction, achieved the synthesis of (1*R*,2*S*)- and (1*R*,2*R*)-c_6Ser and these amino acids were further incorporated into peptides[9] (Fig. 1).

Particularly, (1*R*,2*S*)-c_6Ser was used to synthesise a Leu-enkephalin analogue that behaves as a potent agonist of δ-opioid receptors (10 times more potent than the native Leu-enkephalin).[10]

More recently, Frahm and co-workers reported a new synthesis of the four stereoisomers of c_6Ser starting from 2-methoxycyclohexanone and (*S*)- or (*R*)-1-phenylethylamine via an asymmetric Strecker synthesis.[11]

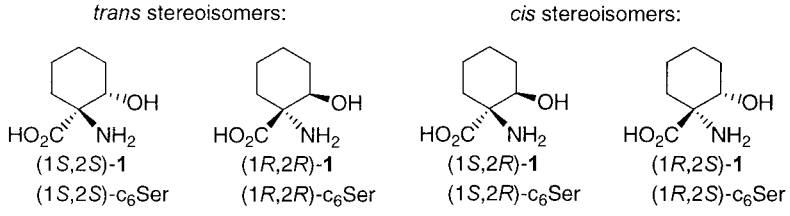

Figure 1. Structures of the four stereoisomers of 1-amino-2-hydroxycyclohexanecarboxylic acid (c_6Ser).

Keywords: Diels–Alder reactions; cyclohexanes; amino acids and derivatives; resolution.
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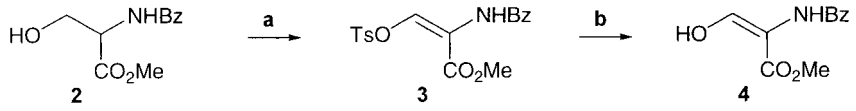
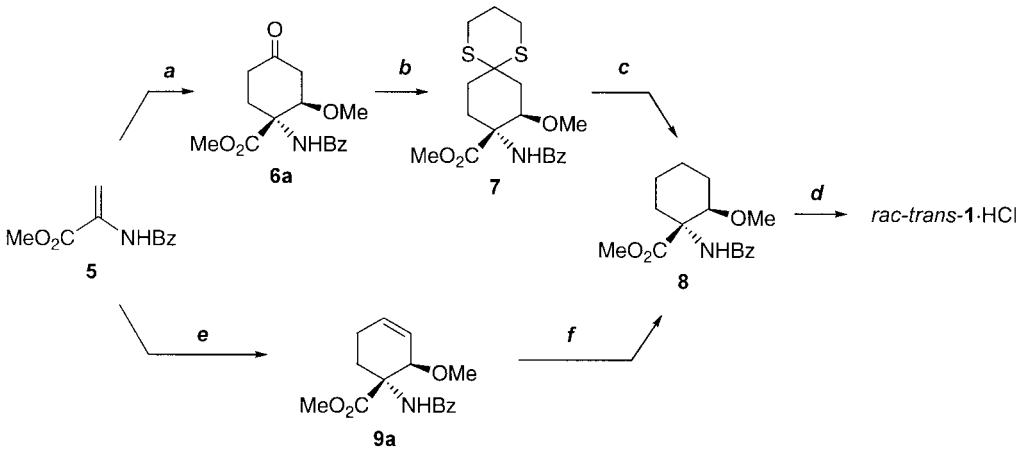


Scheme 1. *Reagents and conditions*: (a) TsCl, DMSO–DMF (1:1), TEA, −5 to 25°C, 1 h, 56%; (b) 1,3-butadiene, $TiCl_4$, CH_2Cl_2, 5–25°C, 48 h, 62%.

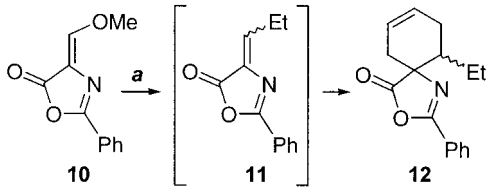


Scheme 2. *Reagents and conditions*: (a) (i) Danishefsky's diene, dioxane, reflux, 6 d; (ii) NaF, THF–H_2O (4:1), room temperature, 15 h; (iii) column chromatography, 48%; (b) 1,3-propanedithiol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, room temperature, 36 h, 77%; (c) Ni-Raney, H_2, EtOH, room temperature, 1.5 h, 62%; (d) 12N HCl, 100°C, 7 d, 84%; (e) 1-methoxy-1,3-butadiene, toluene, 85°C, 6 d, 72%; (f) H_2/Pd–C, MeOH, room temperature, 6 h, 99%.

As part of our research work towards the synthesis of conformationally constrained α-amino acids with a cyclohexane skeleton, in particular analogues of phenylalanine (Phe), we have previously reported the synthesis[12–14] of the four stereoisomers of 1-amino-2-phenylcyclohexanecarboxylic acid (c_6Phe) and their use as conformational probes.[15,16] Moreover, we have recently obtained 3- and 4-hydroxycyclohexane-α-amino acids as a new family of constrained hydroxy-α-amino acids, using a methodology that involves the Diels–Alder reaction as a key step.[17–19] We herein report the extension of this methodology to the synthesis of all stereoisomers of c_6Ser, with the aim of contributing to the development of hydroxylated cyclohexane-α-amino acids, analogues of naturally occurring α-amino acids.

2. Results and discussion

In this paper we present a very efficient strategy for the synthesis and resolution of racemic *trans*- and *cis*-c_6Ser. The resolution method is based on the formation of the corresponding diastereoisomers, which are easily separated by column chromatography. Once separated, these diastereoisomers are selectively cleaved to give, in high yield, the four enantiomerically pure c_6Ser isomers.



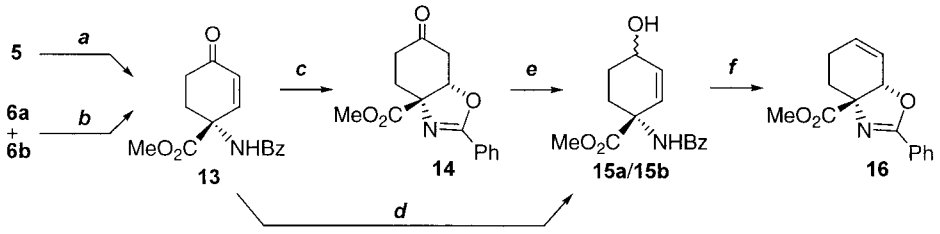
Scheme 3. *Reagents and conditions*: (a) 1,3-butadiene, $AlClEt_2$, CH_2Cl_2, room temperature, 4 d, 15%.

2.1. Synthesis of racemic *trans*-c_6Ser

Initially, in order to obtain the racemic *trans*-c_6Ser **1** and with the similar idea previously described for the synthesis of c_6Phe,[12–14] we assayed the Diels–Alder reaction of 1,3-butadiene with the dienophile **3**, which is easily available from racemic methyl *N*-benzoylserinate **2** using a modified procedure described in the literature.[20] After testing several conditions, reaction was only observed when $TiCl_4$ was used as a catalyst, obtaining the product **4** (Scheme 1).

We decided to change the strategy, introducing the hydroxyl group in the cyclohexane ring from the diene instead of using oxygenated dienophiles. In the course of our research on hydroxy-α,α-disubstituted-α-amino acids, we had demonstrated that methyl 2-benzamidoacrylate **5** behaves as an excellent dienophile with Danishefsky's diene in the Diels–Alder cycloaddition.[21] We are now interested in the synthesis of the *trans*-methoxycyclohexanone **6a**, a pivotal product in the synthesis of racemic *trans*-c_6Ser **1**. As a result of the Diels–Alder reaction, a mixture of two cycloadducts was obtained: methyl 1-benzamido-*c*-2-methoxy-4-trimethylsilyloxy-3-cyclohexene-*r*-1-carboxylate and methyl 1-benzamido-*t*-2-methoxy-4-trimethylsilyloxy-3-cyclohexene-*r*-1-carboxylate, corresponding to *endo* and *exo* attack respectively. This mixture of products was treated with NaF in the presence of THF/H_2O to give a mixture of the corresponding *trans*- and *cis*-methoxycyclohexanones **6a**/**6b** in a ratio 90:10 (Scheme 2).

Ketone **6a** was treated with 1,3-propanedithiol in the presence of $BF_3{\cdot}OEt_2$ as a catalyst to give the corresponding spirothioacetal **7**. Desulphurisation of **7** by hydrogenolysis with Raney nickel gave the 2-methoxycyclohexane derivative **8**, the direct precursor of the amino acid required. The hydrolysis of derivative **8** was carried out in an acid



Scheme 4. *Reagents and conditions*: (a) (i) Danishefsky's diene, toluene, reflux, 72 h; (ii) 0.005N HCl–THF (1:4), room temperature, 15 h; (iii) DBU, CH_2Cl_2, 2°C, 24 h, 55% (b) DBU, CH_2Cl_2, 5°C, 24 h, 96%; (c) TMSTfO, CH_2Cl_2, room temperature, 1 h, 100%; (d) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, room temperature, 10 min, 83%; (e) $NaBH_4$, THF, −10°C, 30 min, 62%; (f) MsCl, TEA, CH_2Cl_2, room temperature, 14 h, 95%.

medium, at reflux, and the cyclic α-hydroxy-α-amino acid *rac-trans*-**1** was obtained as a hydrochloride derivative in a 22% overall yield using four steps (Scheme 2). In order to improve this yield, alternatively, compound **8** was obtained in two steps from the same dienophile **5** by the Diels–Alder reaction with 1-methoxy-1,3-butadiene and further hydrogenation of **9a**. The Diels–Alder reaction produced two stereoisomers, corresponding to *endo*-selectivity: the 1,2-adduct **9a** and the 1,3-adduct **9b** in a ratio 90:10 in favour of **9a**. The *exo*-adducts were not observed by NMR techniques. This second path was optimised on a multigram scale (4 g of starting material **5**) and now, the overall yield of *rac-trans*-c_6Ser HCl was 59% in three steps (Scheme 2).

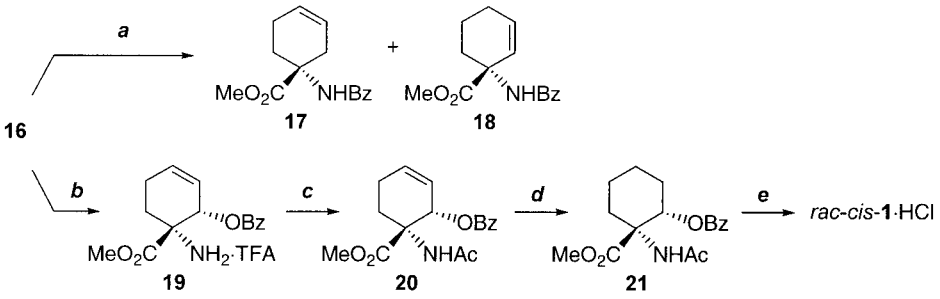
2.2. Synthesis of racemic *cis*-c_6Ser

To obtain *rac-cis*-c_6Ser and taking into account the excellent behaviour of 4-aryliden-2-phenyl-5(4*H*)-oxazolones as dienophiles in the Diels–Alder reactions with several dienes,[22,23] we firstly assayed the cycloaddition of (*Z*)-4-methoxymethylene-2-phenyl-5(4*H*)-oxazolone **10** with 1,3-butadiene. After testing several conditions, reaction was only observed when $AlClEt_2$ was used as a catalyst, obtaining the cycloaddition product **12** in a low yield (Scheme 3).

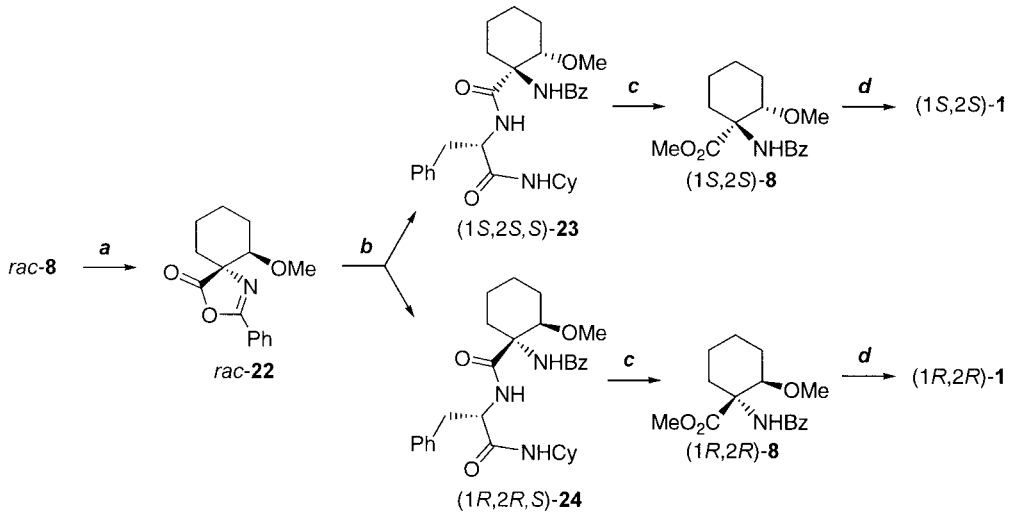
In this case, in order to obtain the corresponding *cis*-β-hydroxycyclohexane-α-amino acid, we introduced the hydroxyl group into the cyclohexane ring using an efficient and easily applicable method,[24] which involves the stereoselective intramolecular conjugate addition of the benzamide group to cyclohexenone **13**. This enone was easily available from the mixture of the Diels–Alder cycloadducts **6a** and **6b**, by elimination of both methoxy groups using DBU in MeOH at 5°C. Alternatively, enone **13** was directly obtained from dienophile **5**, without purification of methoxy cycloadducts **6a** and **6b** and carrying out the transformation of the corresponding silyl enol ethers into ketones by treatment with an aqueous solution of HCl (Scheme 4).

The hydroxy-functionalisation on this enone **13** took place with a very good yield when trimethylsilyl triflate (TMSTfO) were used as a Lewis acid, allowing the direct hydroxylation in a *syn* relationship to the amide group through the oxazoline intermediate **14**. All typical attempts to transform the carbonyl group into methylene failed, obtaining the starting material. Because of this, we assayed the reduction of carbonyl group of oxazoline **14** with the aim to carry out a dehydroxylation. Nevertheless, the reduction of the carbonyl group of oxazoline **14** with $NaBH_4$ produced a mixture of allylic alcohols **15a/15b** instead of the oxazoline alcohols. Better yield of this mixture of alcohols was obtained by reduction of enone **13** with $NaBH_4$ in the presence of $CeCl_3{\cdot}7H_2O$. Treatment of this mixture of alcohols **15a/15b** with methanesulfonyl chloride (MsCl) in triethylamine (TEA) and further purification by silica gel column chromatography allowed us to obtain the unsaturated oxazoline **16**, instead of the corresponding methanesulphonate derivatives. Compound **16** comes from allylic nucleophilic substitution of benzamide group on the methanesulphonate intermediates formed in situ (Scheme 4).

Oxazoline **16** was used as precursor of *rac-cis*-c_6Ser, so we initially tried its hydrogenation and both with palladium and with platinum we obtained a mixture of products (**17** and **18**), derived from hydrogenolysis reaction **17** and a further isomerisation **18**, instead of hydrogenation products[25,26] (Scheme 5). Because of this, we carried out first the hydrolysis of oxazoline ring with trifluoroacetic acid (TFA), to obtain compound **19**, which could be acetylated to afford compound **20**. The hydrogenation of **20** in the



Scheme 5. *Reagents and conditions*: (a) H_2/Pd–C (or Pt–C), CH_2Cl_2, 30°C, 4 h, 92%; (b) TFA, THF–H_2O (4:1), 50°C, 14 h, 95%; (c) AcCl, TEA, CH_2Cl_2, room temperature, 14 h, 80%; (d) H_2/Pd–C, CH_2Cl_2, 35°C, 14 h, 78%; (e) 6N HCl, 100°C, 24 h, 76%.

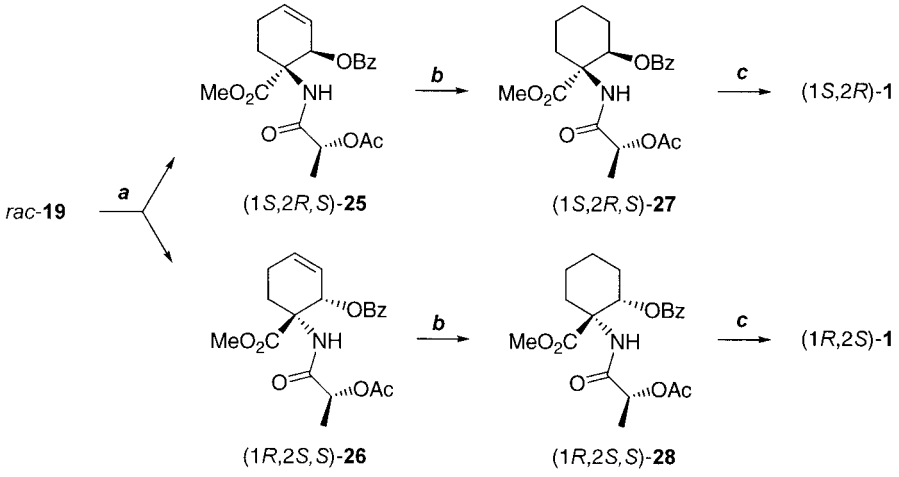


Scheme 6. *Reagents and conditions*: (a) (i) $LiOH{\cdot}H_2O$, MeOH–H_2O (3:2), reflux, 7 h, 93%; (ii) DCC, DMAP, CH_2Cl_2, room temperature, 3 h, 91%; (b) L-phenylalanine cyclohexylamide, NMP, 90°C, 48 h, column chromatography: 36% of **23**, 35% of **24**; (c) TfOH, MeOH, 80°C, 48 h, 91%; (d) (i) 12N HCl, 100°C, 7 d, 90%; (ii) propylene oxide, EtOH, reflux, 2 h, 83%.

presence of platinum–carbon as a catalyst worked without problems to give compound **21**, which is the direct precursor of *rac-cis*-c_6Ser, so its hydrolysis gave with an excellent yield the required *rac-cis*-**1** as a hydrochloride derivative. In this way, *rac-cis*-c_6Ser·HCl was obtained from **5** on a multigram scale with a 16% yield, in seven steps (Scheme 5).

2.3. Synthesis of (1*S*,2*S*)- and (1*R*,2*R*)-c_6Ser

The strategy used to resolve the racemic *trans*-c_6Ser **1** was previously developed and reported by Obrecht to prepare and resolve both cyclic and acyclic *N*-acylated α,α-disubstituted amino acids.[27–29] In our case, the synthesis of the two enantiomerically pure amino acids (1*S*,2*S*)-**1** and (1*R*,2*R*)-**1** started from *rac*-**8** (Scheme 6). The methyl ester group of *rac*-**8** was hydrolysed by the action of LiOH and further treatment of the corresponding carboxylic acid with *N*,*N*-dicyclohexylcarbodiimide (DCC) in the presence of 4-dimethylaminopyridine (DMAP) gave the spirooxazolone *rac*-**22**. This compound was smoothly reacted with L-phenylalanine cyclohexylamide in *N*-methylpyrrolidin-2-one (NMP) as a solvent, at 90°C. The corresponding diastereoisomeric peptides (1*S*,2*S*,*S*)-**23** and (1*R*,2*R*,*S*)-**24** were obtained in good yields after column chromatography using toluene–ethyl acetate (1:1) as an eluent. Each diastereoisomeric peptide **23** and **24** was separately treated with trifluoromethanesulphonic acid (TfOH) in MeOH at 80°C to give the optically pure methyl esters (1*S*,2*S*)-**8** and (1*R*,2*R*)-**8**. Finally, the concomitant hydrolysis of the benzamide, methyl ester and methyl ether groups, in the same conditions as described above for *rac*-**8**, gave the optically pure (1*S*,2*S*)- and (1*R*,2*R*)-c_6Ser as hydrochloride derivatives. In order to assess the enantiomeric purity and to determine the absolute configuration of each amino acid, each hydrochloride derivative was dissolved in EtOH and propylene oxide was then added. After 2 h at reflux, the free amino acids (1*S*,2*S*)-**1** and (1*R*,2*R*)-**1** were obtained and the observed optical rotations were in agreement with those described in the literature[11] (Scheme 6).



Scheme 7. *Reagents and conditions*: (a) (*S*)-2-acetoxypropionyl chloride, TEA, CH_2Cl_2, room temperature, 2 h, column chromatography: 40% of **25**, 50% of **26**; (b) H_2/Pt–C, ethyl acetate, room temperature, 3 h, 99%; (c) (i) 9N HCl, 85°C, 4 d, 93%; (ii) propylene oxide, EtOH, reflux, 2 h, 85%.

2.4. Synthesis of (1*S*,2*R*)- and (1*R*,2*S*)-c$_6$Ser

On the other hand, the strategy used to resolve the racemic *cis*-c$_6$Ser **1** involves the reaction of *rac*-**19** with (*S*)-2-acetoxypropanoyl chloride in the presence of TEA to give with 90% yield a diastereomeric mixture of (1*S*,2*R*,*S*)-**25** and (1*R*,2*S*,*S*)-**26**. These diastereoisomers were separated by column chromatography using ethyl ether–hexane (7:3) as an eluent. Each diastereoisomer **25** and **26** was separately hydrogenated in the presence of platinum–carbon as a catalyst and ethyl acetate as a solvent to give, respectively, the compounds (1*S*,2*R*,*S*)-**27** and (1*R*,2*S*,*S*)-**28**, which were subjected to hydrolysis using 6N HCl at reflux to obtain (1*S*,2*R*)- and (1*R*,2*S*)-c$_6$Ser as hydrochloride derivatives. In the same way described for optically active *trans*-c$_6$Ser, we obtained the free amino acids (1*S*,2*R*)-**1** and (1*R*,2*S*)-**1** in order to assess their enantiomeric purity and to determine their absolute configuration (Scheme 7).

3. Conclusions

In summary, we have developed a new methodology for the synthesis, on a multigram scale, of two types of racemic constrained serines (*trans*- and *cis*-c$_6$Ser) from methyl 2-benzamidoacrylate, using as a key step the Diels–Alder reaction with oxygenated dienes. Moreover, we have prepared, in a good yield, all four enantiomerically pure amino acids (1*S,2S*)-, (1*R,2R*)-, (1*S,2R*)- and (1*R,2S*)-c$_6$Ser by resolution of the corresponding diastereoisomers. In a future work, we will introduce these amino acids in small peptides as restricted serine analogues and we will make several structural studies.

4. Experimental

4.1. General procedure

Solvents were purified according to standard procedures. Analytical TLC was performed using Polychrom SI F_{254} plates. Column chromatography was performed using silica gel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 spectrometer at 300 MHz (^{1}H) and at 75 MHz (^{13}C) in $CDCl_3$ and CD_3OD with TMS as the internal standard and in D_2O with TMS as the external standard using a coaxial microtube (chemical shifts are reported in ppm on the δ scale, coupling constants in Hz). Melting points were determined on a Büchi SMP-20 melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 polarimeter in a 1 dm cell of 1 mL capacity. Microanalyses were carried out on a CE Instruments EA-1110 analyser and were in good agreement with the calculated values. IR spectra were recorded on a Perkin Elmer FT-IR Spectrum 1000 spectrometer.

4.1.1. Methyl (*E*)-2-benzamido-3-(*p*-toluenesulphonyloxy)-2-propenoate (3). *p*-Toluenesulphonyl chloride (718 mg, 3.7 mmol) was dissolved in a mixture of DMSO–DMF 1:1 (15 mL) at −5°C and a solution of methyl *N*-benzoylserinate (280 mg, 1.2 mmol) in dry DMF (10 mL) was added dropwise, under an inert atmosphere. After stirring for 5 min at the same temperature, Et_3N (1.26 g, 12.5 mmol) was added and the mixture was allowed to warm up to 25°C. After stirring for 1 h, the reaction was quenched by the addition of water (25 mL). The aqueous solution was extracted with CH_2Cl_2 (3×20 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel, eluting with hexane–ethyl acetate (6:4), to yield 263 mg of compound **3** as a white solid (56%). Mp: 129–130°C; Anal. calcd for $C_{18}H_{17}NO_6S$: C, 57.59; H, 4.56; N, 3.73; S, 8.54; found: C, 57.30; H, 4.55; N, 3.64; S, 8.68; IR (CH_2Cl_2, cm^{-1}): 3418 (NH), 1728 (COO), 1686 (CON); ^{1}H NMR ($CDCl_3$): δ 2.44 (s, 3H, CH_3Ph); 3.78 (s, 3H, CH_3O); 7.36–7.46 (m, 4H, Arom.); 7.47–7.53 (m, 2H, Arom.+NH); 7.54 (s, 1H, H_3); 7.76–7.81 (m, 2H, Arom.); 7.85 (d, 2H, *J*=8.4 Hz, Arom.); ^{13}C NMR ($CDCl_3$): δ 21.7 (CH_3Ph); 52.6 (CH_3O); 116.0 (C_2); 127.5, 128.2, 128.6, 130.2, 131.8, 132.2, 133.0, 138.4 (Arom.); 146.3 (C_3); 163.7, 165.0 (COO, CON).

4.1.2. Methyl (*E*)-2-benzamido-3-hydroxy-2-propenoate (4). A 1 M solution of $TiCl_4$ in CH_2Cl_2 (0.53 mL, 0.53 mmol) was added to a solution of dienophile **3** (200 mg, 0.53 mmol) in dry CH_2Cl_2 (10 mL) at 5°C, under an inert atmosphere. After stirring at the same temperature for 1 h, a solution of 1,3-butadiene (286 mg, 5.3 mmol) in dry CH_2Cl_2 (2 mL) was added and the mixture was allowed to warm up to 25°C. After stirring for 48 h, the reaction was quenched by the addition of solid $Na_2CO_3{\cdot}10H_2O$, filtered and evaporated. The residue was chromatographed on silica gel, eluting with hexane–ethyl acetate (1:1), to yield 75 mg of compound **4** as an oil (62%). Anal. calcd for $C_{11}H_{11}NO_4$: C, 59.73; H, 5.01; N, 6.33; found: C, 60.03; H, 5.11; N, 6.21; IR (CH_2Cl_2, cm^{-1}): 3418 (NH+OH), 1737 (COO), 1686 (CON); ^{1}H NMR ($CDCl_3$): δ 3.83 (s, 3H, CH_3); 7.05 (s, 1H, H_3); 7.44–7.51 (m, 3H, Arom.+OH); 7.53–7.62 (m, 2H, Arom.+NH); 7.86–7.91 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 52.9 (CH_3); 123.2 (C_2); 127.6 (Arom.); 128.8 (Arom.+C_3); 132.6 (Arom.); 162.9, 165.1 (COO, CON).

4.1.3. Methyl 1-benzamido-*c*-2-methoxy-4-oxocyclohexane-*r*-1-carboxylate (6a) and methyl 1-benzamido-*t*-2-methoxy-4-oxocyclohexane-*r*-1-carboxylate (6b). Danishefsky's diene (1 g, 6.0 mmol) was added to a solution of methyl 2-benzamidoacrylate **5** (615 mg, 3.0 mmol) in dry dioxane (25 mL) under an inert atmosphere. After stirring at reflux for 24 h, another 3.0 mmol of **5** were added. The reaction was stirred for another 24 h and then another 6.0 mmol of Danishefsky's diene were added. After stirring at reflux for 6 d overall, the solvent was evaporated to give a residue corresponding to a mixture of silyl enol ethers. This mixture (2.1 g, 5.6 mmol) was dissolved in a mixture of THF–H_2O (4:1) (50 mL) and NaF (517 mg, 12 mmol) was added. After stirring at 25°C for 15 h, the reaction was extracted with CH_2Cl_2 (3×50 mL) and the combined organic phases were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica gel, eluting with hexane–ethyl acetate (4:6), to yield 875 mg of ketone **6a** (48%) and 73 mg of ketone **6b** (4%) as white solids.

6a. Mp: 128–129°C; Anal. calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59; found: C, 63.09; H, 6.22; N, 4.53; IR (CH_2Cl_2, cm^{-1}): 3414 (NH), 1720 (CO, COO), 1673 (CON); ^{1}H NMR

($CDCl_3$): δ 2.41–2.82 (m, 6H, $2H_3+2H_5+2H_6$); 3.30 (s, 3H, CH_3O); 3.79 (s, 3H, CH_3O_2C); 3.98 (t, 1H, $J_{2e-3a}=J_{2e-3e}=$ 5.2 Hz, H_{2e}); 7.02 (br s, 1H, NH); 7.37–7.53 (m, 3H, Arom.); 7.73–7.81 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 26.3, 36.9, 41.2 (C_3, C_5, C_6); 52.9 (CH_3O_2C); 57.7 (CH_3O); 62.0 (C_1); 80.1 (C_2); 127.1, 128.6, 132.1, 133.9 (Arom.); 167.7, 172.0 (COO, CON); 208.1 (CO).

6b. Mp: 134–136°C; Anal. calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59; found: C, 63.12; H, 6.24; N, 4.64; IR (CH_2Cl_2, cm^{-1}): 3426 (NH), 1744, 1720 (CO, COO), 1677 (CON); ^1H NMR ($CDCl_3$): δ2.35–2.94 (m, 6H, $2H_3+2H_5+2H_6$); 3.41 (s, 3H, CH_3O); 3.82 (s, 3H, CH_3O_2C); 4.05 (dd, 1H, $J_{2a-3a}=7.5$ Hz; $J_{2a-3e}=4.2$ Hz, H_{2a}); 6.80 (br s, 1H, NH); 7.39–7.59 (m, 3H, Arom.); 7.74–7.84 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 27.5, 36.6, 41.8 (C_3, C_5, C_6); 53.0 (CH_3O_2C); 57.3 (CH_3O); 62.1 (C_1); 80.8 (C_2); 126.9, 128.6, 131.9, 133.9 (Arom.); 168.0, 171.8 (COO, CON); 206.9 (CO).

4.1.4. Methyl 1-benzamido-*c*-2-methoxy-4-spiro-[2′ (1′,3′-dithiocyclohexane)]cyclohexane-*r*-1-carboxylate (7). 1,3-Propanedithiol (89 mg, 0.82 mmol) was added to a solution of ketone **6a** (200 mg, 0.65 mmol) in dry CH_2Cl_2 (20 mL) at 25°C, under an inert atmosphere. After stirring for 30 min, $BF_3{\cdot}OEt_2$ (13 mg, 0.09 mmol) was added and the mixture was stirred for another 36 h at the same temperature. The reaction was quenched by the addition of solid $Na_2CO_3{\cdot}10H_2O$, filtered and evaporated. The residue was chromatographed on silica gel eluting with hexane–ethyl acetate (1:1), to yield 200 mg of compound **7** as a white solid (77%). Mp: 136–138°C; Anal. calcd for $C_{19}H_{25}NO_4S_2$: C, 57.69; H, 6.37; N, 3.54; S, 16.21; found: C, 57.81; H, 6.32; N, 3.62; S, 16.07; IR (CH_2Cl_2, cm^{-1}): 3410 (NH), 1731 (COO), 1670 (CON); ^1H NMR ($CDCl_3$): δ1.93–2.08 (m, 2H); 2.17–2.39 (m, 3H); 2.40–2.64 (m, 2H); 2.75–3.02 (m, 5H); 3.39 (s, 3H, CH_3O); 3.82 (s, 3H, CH_3O_2C); 4.41 (dd, 1H, $J_{2a-3a}=10.2$ Hz, $J_{2a-3e}=4.5$ Hz, H_{2a}); 7.20 (br s, 1H, NH); 7.38–7.56 (m, 3H, Arom.); 7.76–7.85 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 25.5, 26.1, 26.4, 27.1, 33.5, 37.4 (C_3, C_5, C_6, $C_{4'}$, $C_{5'}$, $C_{6'}$); 48.4 (C_4); 52.7 (CH_3O_2C); 58.0 (CH_3O); 63.9 (C_1); 76.9 (C_2); 126.8, 128.4, 131.5, 134.5 (Arom.); 166.7, 171.9 (COO, CON).

4.1.5. Methyl 1-benzamido-*c*-2-methoxy-3-cyclohexene-*r*-1-carboxylate (9a) and methyl 1-benzamido-*c*-3-methoxy-4-cyclohexene-*r*-1-carboxylate (9b). 1-Methoxy-1,3-butadiene (4 g, 19.52 mmol) and hydroquinone (10 mg) were added to a solution of methyl 2-benzamidoacrylate **5** (4 g, 47.62 mmol) in toluene (40 mL) at 85°C. After stirring for 6 d, the reaction was allowed to cool down to 25°C. The formed precipitate was filtered and washed with cold diethyl ether (2×30 mL), to yield 3.22 g of compound **9a** as a white solid (57%). The filtrate was evaporated and the residue was chromatographed on silica gel eluting with hexane–ethyl acetate (6:4), to yield a further 870 mg of compound **9a** (15, 72% overall) and 524 mg of compound **9b** as an oil (9%).

9a. Mp: 148–150°C; Anal. calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84; found: C, 66.65; H, 6.68; N, 4.99; IR (CH_2Cl_2, cm^{-1}): 3433 (NH), 1752 (COO), 1670 (CON); ^1H NMR ($CDCl_3$): δ 1.87–2.21 (m, 3H); 2.48–2.57 (m, 1H); 3.32 (s, 3H, CH_3O); 3.73 (s, 4H, $CH_3O_2C+H_2$); 5.85–5.93 (m, 1H, H_3); 6.02–6.13 (m, 2H, H_4+NH); 7.30–7.46 (m, 3H, Arom.); 7.60–7.67 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 21.5 (C_6); 22.6 (C_5); 52.3 (CH_3O_2C); 57.4 (CH_3O); 60.7 (C_1); 76.5 (C_2); 121.9 (C_3); 126.9, 128.5, 131.8, 133.6 (Arom.); 134.7 (C_4); 166.5 (CON); 172.2 (COO).

9b. Anal. calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84; found: C, 66.73; H, 6.78; N, 4.99; IR (CH_2Cl_2, cm^{-1}): 3425 (NH), 1731 (COO), 1669 (CON); ^1H NMR ($CDCl_3$): δ 1.98–2.17 (m, 3H); 2.89–2.97 (m, 1H); 3.39 (s, 3H, CH_3O); 3.75 (s, 3H, CH_3O_2C); 4.17–4.24 (m, 1H, H_3); 5.64 (dd, 1H, $J_{4-5}=10.2$ Hz, $J_{4-3}=1.5$ Hz, H_4); 5.93–5.99 (m, 1H, H_5); 6.73 (br s, 1H, NH); 7.29–7.53 (m, 3H, Arom.); 7.71–7.80 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 22.1 (C_6); 26.5 (C_2); 52.6 (CH_3O_2C); 57.7 (CH_3O); 60.9 (C_1); 78.8 (C_3); 123.5 (C_4); 126.9, 128.3 (Arom.); 130.9 (C_5); 131.4, 134.4 (Arom.); 167.7 (CON); 173.0 (COO).

4.1.6. Methyl 1-benzamido-*c*-2-methoxycyclohexane-*r*-1-carboxylate (*rac*-8). *Method A*: A suspension of Raney Ni catalyst in water (5 mL) was added to a solution of compound **7** (220 mg, 0.56 mmol) in EtOH (30 mL). The mixture was hydrogenated at atmospheric pressure, vigorously stirring at 25°C for 90 min. The catalyst was filtered off through Celite, washed with EtOH (3×10 mL) and the filtrate evaporated. The residue was chromatographed on silica gel eluting with hexane–ethyl acetate (6:4), to yield 101 mg of compound **8** as a white solid (62%). *Method B*: A solution of compound **9a** (1.0 g, 3.46 mmol) in MeOH (30 mL) was hydrogenated at atmospheric pressure, using palladium–carbon (10%) as a catalyst (100 mg), vigorously stirring at 25°C for 6 h. The catalyst was filtered off through Celite and the solvent evaporated to yield 1 g of compound **8** as a white solid (99%). Mp: 132–133°C; Anal. calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81; found: C, 65.84; H, 7.32; N, 4.75; IR ($CHCl_3$, cm^{-1}): 3411 (NH), 1729 (COO), 1664 (CON); ^1H NMR ($CDCl_3$): δ 1.38–1.51 (m, 2H); 1.55–1.78 (m, 3H); 1.84–1.99 (m, 1H); 2.04–2.18 (m, 1H); 2.39–2.51 (m, 1H); 3.31 (s, 3H, CH_3O); 3.78 (s, 3H, CH_3O_2C); 3.76–3.91 (m, 1H, H_2); 6.68 (br s, 1H, NH); 7.37–7.56 (m, 3H, Arom.); 7.71–7.82 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 20.7, 21.3, 25.3, 27.8 (C_3, C_4, C_5, C_6); 52.5 (CH_3O_2C); 57.5 (CH_3O); 62.9 (C_1); 79.5 (C_2); 126.9, 128.6, 131.6, 134.5 (Arom.); 166.6, 173.1 (COO, CON).

4.1.7. 1-Amino-*c*-2-hydroxycyclohexane-*r*-1-carboxylic acid (*rac-trans*-1). Compound *rac*-**8** (2 g, 6.87 mmol) was suspended in a 12N HCl solution (25 mL). After stirring under reflux for 7 d, the solvent was evaporated, the excess of HCl removed in vacuo and the residue was dissolved in distilled water (20 mL). The aqueous mixture was washed with diethyl ether (4×20 mL) and evaporated to yield 1.2 g of *rac-trans*-**1**·HCl as a white solid (91%). The hydrochloride was dissolved in ethanol (30 mL) and propylene oxide (10 mL) was added. After stirring under reflux for 2 h, the precipitate was filtered off and washed with cold EtOH to yield 819 mg of *rac-trans*-**1** as a white solid (75%). The filtrate was evaporated and the residue was

dissolved in distilled water (5 mL) and eluted through a C_{18} reverse-phase Sep-pak cartridge which, after removal of water, gave another 105 mg (9%, 84% overall) of the amino acid. *rac-trans*-**1**·HCl: ^{1}H NMR (CD_3OD):δ 1.41–1.49 (m, 1H); 1.58–1.71 (m, 2H); 1.75–1.92 (m, 3H); 1.99–2.18 (m, 2H); 3.72 (dd, 1H, J_{2a-3a}=11.1 Hz, J_{2a-3e}=4.8 Hz, H_{2a}); ^{13}C NMR (CD_3OD): δ 22.1, 24.4, 31.3, 32.9 (C_3, C_4, C_5, C_6); 65.1 (C_1); 73.8 (C_2); 171.6 (COO). *rac-trans*-**1**: Anal. calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80; found: C, 52.65; H, 8.36; N, 8.67; ^{1}H NMR (D_2O): δ 1.22–1.39 (m, 1H); 1.42–1.99 (m, 6H); 2.04–2.18 (m, 1H); 3.63 (dd, 1H, J_{2a-3a}=10.5 Hz, J_{2a-3e}=4.8 Hz, H_{2a}); ^{13}C NMR (D_2O): δ 21.0, 22.7, 29.8, 31.5 (C_3, C_4, C_5, C_6); 64.1 (C_1); 72.5 (C_2); 174.0 (COO).

4.1.8. (*cis* and *trans*)-6-Ethyl-3-cyclohexene-1-spiro-{4′-[2′-phenyl-5′(4′H)-oxazolone]} (12). A 1 M solution of $AlClEt_2$ in hexane (4.20 mL, 4.20 mmol) was added to a solution of oxazolone **10** (850 mg, 4.20 mmol) in dry CH_2Cl_2 (25 mL) at 25°C, under an inert atmosphere. After stirring for 1 h, a solution of 1,3-butadiene (2 g, 37.0 mmol) in dry CH_2Cl_2 (2 ml) was added to the mixture. After stirring for 48 h, another 37.0 mmol of 1,3-butadiene were added. After stirring for another 48 h at the same temperature, the reaction was quenched by the addition of solid $Na_2CO_3·10H_2O$, filtered and evaporated. The residue was chromatographed on silica gel eluting with hexane–ethyl acetate (8:2), to yield 166 mg of the mixture of oxazolones **12** as a yellow oil (15%). ^{1}H NMR ($CDCl_3$): δ 0.78–0.93 (m, CH_2CH_3); 0.93–1.10, 1.15–1.34 (m, CH_2CH_3); 1.97–2.29, 2.31–2.50, 2.57–2.78 (m, H_2, H_5, H_6); 5.61–5.77; 5.85–5.98 (m, H_3, H_4); 7.40–7.62 (m, Arom.); 7.95–8.10 (m, Arom.); ^{13}C NMR ($CDCl_3$): δ 11.2, 11.4 (CH_2CH_3); 22.6, 23.5, 26.4, 27.1, 33.6, 35.3, 40.7, 40.8 (C_2, C_5, C_6, CH_2CH_3); 70.2, 72.1 (C_1); 120.9, 121.9, 125.8, 125.9, 126.9, 127.2, 127.9, 128.0, 128.7, 128.8, 132.5, 132.6 (C_3, C_4, Arom.); 160.3 (CON); 181.5 (COO).

4.1.9. Methyl 1-benzamido-4-oxo-2-cyclohexene-1-carboxylate (13). *Method A*: Danishefsky's diene (6.5 mL, 33.4 mmol) was added to a solution of methyl 2-benzamidoacrylate **5** (1.7 g, 8.3 mmol) in dry toluene (80 mL) under an inert atmosphere. After stirring at reflux for 24 h, another 8.3 mmol of Danishefsky's diene were added. After stirring for another 2 d at the same temperature, the solvent was evaporated in vacuo and a 0.005N HCl–THF (1:4) solution (40 mL) was added to the residue. The reaction mixture was stirred for 15 h at 20°C, the solvent was evaporated and the residue was chromatographed on silica gel, eluting with hexane–ethyl acetate (2:8). The mixture of compounds **6a**, **6b** and **13** was dissolved in CH_2Cl_2 (75 mL) and DBU (2.7 mL, 18.2 mmol) was added. The reaction mixture was stirred for 24 h at 2°C and the solution was washed with 0.5N HCl (60 mL). The aqueous phase was extracted with CH_2Cl_2 (5×30 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel eluting with hexane–ethyl acetate (1:1), to yield 2.5 g of enone **13** as a white solid (55%). *Method B*: A mixture of ketones **6a** and **6b** in a 90:10 ratio (500 mg, 1.63 mmol) was dissolved in CH_2Cl_2 (30 mL) and DBU (0.27 mL, 1.80 mmol) was added. The reaction mixture was stirred for 24 h at 5°C and the solution was washed with a saturated $NaHCO_3$ solution (3×20 mL). The aqueous phase was extracted with CH_2Cl_2 (20 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel eluting with hexane–ethyl acetate (1:1), to yield 429 mg of enone **13** as a white solid (96%). Mp: 126–127°C; Anal. calcd for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13; found: C, 65.84; H, 5.59; N, 5.05; IR (CH_2Cl_2, cm^{-1}): 3433 (NH), 1744 (CO), 1673 (CON); ^{1}H NMR ($CDCl_3$): δ 2.50–2.65 (m, 4H, $2H_5$+$2H_6$); 3.82 (s, 3H, CH_3); 6.14 (d, 1H, J_{3-2}=10.0 Hz, H_3); 6.98 (br s, 1H, NH); 7.14 (d, 1H, J_{2-3}=10.0 Hz, H_2); 7.41–7.48 (m, 2H, Arom.); 7.50–7.58 (m, 1H, Arom.); 7.76–7.82 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 31.6, 33.7 (C_5, C_6); 53.4 (CH_3); 58.5 (C_1); 127.1, 128.6, 130.5, 132.2 (C_3, Arom.); 133.0 (Arom.); 146.9 (C_2); 167.0, 171.2 (COO, CON); 197.2 (CO).

4.1.10. Methyl *cis*-8-oxo-3-phenyl-2-oxa-4-azabicyclo-[4.3.0]non-3-ene-5-carboxylate (14). TMSTfO (732 mg, 3.30 mmol) was added dropwise to a solution of enone **13** (300 mg, 1.10 mmol) in dry CH_2Cl_2 (30 mL) at 25°C, under an inert atmosphere. After stirring for 1 h, the reaction was quenched by the addition of solid $Na_2CO_3·10H_2O$, dried over anhydrous Na_2SO_4, filtered and evaporated, to yield 300 mg of compound **14** as an oil (100%). Anal. calcd for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13; found: C, 65.99; H, 5.42; N, 5.08; ^{1}H NMR ($CDCl_3$): δ 2.14–2.51 (m, 4H, $2H_6$+$2H_7$); 2.79 (dd, 1H, J_{9x-9n}=17.4 Hz, J_{9x-1}=3.6 Hz, H_{9x}); 3.00 (dd, 1H, J_{9n-9x}=17.4 Hz, J_{9n-1}=3.0 Hz, H_{9n}); 3.87 (s, 3H, CH_3); 5.43 (t, 1H, J=3.3 Hz, H_1); 7.38–7.47 (m, 2H, Arom.); 7.48–7.56 (m, 1H, Arom.); 7.92–7.98 (m, 2H, Arom.).

4.1.11. Methyl 1-benzamido-*t*-4-hydroxy-2-cyclohexene-*r*-1-carboxylate (15a) and methyl 1-benzamido-*c*-4-hydroxy-2-cyclohexene-*r*-1-carboxylate (15b). *Method A*: A 0.4 M solution of $CeCl_3·7H_2O$ in MeOH (2.56 mL, 1.02 mmol) was added to a solution of enone **13** (280 mg, 1.02 mmol) in MeOH (20 mL) at 25°C. $NaBH_4$ (38.7 mg, 1.02 mmol) was then added to the mixture and after stirring for 10 min at the same temperature, the reaction was quenched by the dropwise addition of a 2N HCl solution. The mixture was extracted with CH_2Cl_2 (3×25 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel, eluting with hexane–ethyl acetate (3:7), to yield 234 mg of the mixture of alcohols **15a/15b** in an approximate ratio of 6:4 as a white solid (83%). *Method B*: $NaBH_4$ (111 mg, 2.9 mmol) was added to a solution of ketone **14** (160 mg, 0.59 mmol) in dry THF (15 mL) at −10°C. After stirring for 30 min at the same temperature, the reaction was quenched by the dropwise addition of water. The mixture was extracted with CH_2Cl_2 (3×15 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel, eluting with hexane–ethyl acetate (3:7), to yield 101 mg of the mixture of alcohols **15a/15b** in an approximate ratio of 7:3, as a white solid (62%). Anal. calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09; found: C, 65.59; H, 6.13; N, 5.08; ^{1}H NMR ($CDCl_3$): δ 1.68–2.51 (m); 3.76 (s, CH_3); 4.16–4.26 (m, H_4+H_4'); 5.79 (d, J_{3-2}=9.9 Hz, H_3); 5.95

(d, $J_{3'-2}$=9.9 Hz, H_3'); 6.06 (dd, $J_{2'-3'}$=9.9 Hz, $J_{2'-4'}$= 3.0 Hz, H_2'); 6.13 (dd, J_{2-3}=9.9 Hz, J_{2-4}=3.7 Hz, H_2); 6.68 (br s, NH); 7.08 (br s, NH′); 7.34–7.53 (m, Arom.); 7.71–7.81 (m, Arom.).

4.1.12. Methyl *cis*-3-phenyl-2-oxa-4-azabicyclo[4.3.0]-nona-3,8-diene-5-carboxylate (16). Et_3N (143 mg, 2.42 mmol) and MsCl (162 mg, 1.42 mmol) were added to a solution of the mixture of alcohols **15a/15b** (300 mg, 1.10 mmol) in dry CH_2Cl_2 (25 mL) at 25°C, under an inert atmosphere. After stirring for 14 h, the mixture was washed with a saturated $NaHCO_3$ solution (2×20 mL) and the organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel, eluting with hexane–ethyl acetate (1:1), to yield 270 mg of compound **16** as an oil (95%). Anal. calcd for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44; found: C, 70.14; H, 5.87; N, 5.35; ^{1}H NMR ($CDCl_3$): δ 1.93–2.32 (m, 4H, $2H_6$+$2H_7$); 3.80 (s, 3H, CH_3); 5.33 (d, 1H, J_{1-9}=3.6 Hz, H_1); 5.94–6.00 (m, 1H, H_9); 6.12–6.20 (m, 1H, H_8); 7.36–7.42 (m, 2H, Arom.); 7.43–7.51 (m, 1H, Arom.); 7.95–8.01 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 20.8, 29.1 (C_6, C_7); 52.5 (CH_3); 74.4 (C_5); 76.7 (C_1); 122.8 (C_9); 127.3, 128.0, 128.2, 131.5 (Arom.); 133.5 (C_8); 164.9, 173.0 (COO, C_3).

4.1.13. Methyl 1-benzamido-3-cyclohexene-1-carboxylate (17) and methyl 1-benzamido-2-cyclohexene-1-carboxylate (18). A solution of compound **16** (150 mg, 0.58 mmol) in dry CH_2Cl_2 (15 mL) was hydrogenated at atmospheric pressure, using palladium–carbon (10%) as a catalyst (30 mg), vigorously stirring at 30°C for 4 h. The catalyst was filtered off through Celite and the solvent evaporated to yield 139 mg of the mixture of compounds **17/18** in a ratio 4:6 as a white solid (92%). Anal. calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40; found: C, 69.55; H, 6.64; N, 5.34; ^{1}H NMR ($CDCl_3$): δ 1.57–2.72 (m, H_4'+ H_5'+H_6'+H_2+H_5+H_6); 3.76 (s, CH_3); 5.61–5.69 (m, H_4); 5.77–5.85 (m, H_3); 5.86–5.93 (m, H_2'); 6.03–6.12 (m, H_3'); 6.40 (br s, 1H, NH); 6.54 (br s, 1H, NH′); 7.33–7.54 (m, Arom.); 7.67–7.81 (m, Arom.); ^{13}C NMR ($CDCl_3$): δ 18.5, 21.8, 24.7, 26.7, 30.3, 34.1 (C_4', C_5', C_6', C_2, C_5, C_6); 52.5, 52.8 (CH_3); 57.1, 58.1 (C_1+C_1'); 122.3, 125.7, 126.9, 127.0, 127.5, 128.5, 131.6, 133.5 (C_3, C_4, C_2', C_3', Arom.); 166.4, 167.0, 173.3, 174.0 (COO, CON).

4.1.14. Methyl 1-amino-*t*-2-benzoyloxy-3-cyclohexene-*r*-1-carboxylate trifluoroacetate (19). Compound **16** (385 mg, 1.50 mmol) was dissolved in THF–H_2O (4:1) (30 mL) and trifluoroacetic acid (855 mg, 7.50 mmol) was then added. After stirring for 14 h at 50°C, the water was removed by the addition of anhydrous Na_2SO_4. The remaining filtrate was then evaporated without warming. The oily residue was then dissolved in Et_2O and the solvent and the residual trifluoroacetic acid were distilled off in vacuo. This operation was repeated to ensure the complete removal of the trifluoroacetic acid, to give 550 mg of trifluoroacetate **19** as a white solid (95%). Anal. calcd for $C_{17}H_{18}F_3NO_6$: C, 52.45; H, 4.66; N, 3.60; found: C, 52.33; H, 4.58; N, 3.78; ^{1}H NMR ($CDCl_3$): δ 2.25–2.50 (m, 4H, $2H_5$+$2H_6$); 3.66 (s, 3H, CH_3); 5.60–5.70 (m, 1H, H_3); 5.92–6.00 (m, 1H, H_4); 6.05 (br s, 1H, H_2); 7.28–7.40 (m, 2H, Arom.); 7.48–7.58 (m, 1H, Arom.); 8.09–8.18 (m, 2H, Arom.); 8.56 (br s, 3H, NH_3); ^{13}C NMR ($CDCl_3$): δ 20.3, 26.8 (C_5, C_6); 53.9 (CH_3); 60.3 (C_1); 70.0 (C_2); 122.7 (C_3); 128.3, 128.4, 130.2, 131.1, 133.7 (Arom, C_4); 165.3, 169.7 (COO, PhCOO).

4.1.15. Methyl 1-acetamido-*t*-2-benzoyloxy-3-cyclohexene-*r*-1-carboxylate (20). Trifluoroacetate **19** (550 mg, 1.41 mmol), Et_3N (186 mg, 1.84 mmol) and acetyl chloride (144 mg, 1.84 mmol) were dissolved in dry CH_2Cl_2 (35 mL) at 25°C, under an inert atmosphere. After stirring for 14 h, the reaction was washed with a saturated $NaHCO_3$ solution (2×25 mL) and the organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel eluting with hexane–ethyl acetate (8:2), to yield 358 mg of compound **20** as a white solid (80%). Mp: 131–132°C; Anal. calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.03; N, 4.41; found: C, 64.02; H, 6.15; N, 4.22; IR (CH_2Cl_2, cm^{-1}): 3441 (NH), 1742, 1727 (COO, PhCOO), 1685 (CON); ^{1}H NMR ($CDCl_3$): δ 2.03 (s, 3H, CH_3CO); 2.09–2.20 (m, 3H); 2.76–2.82 (m, 1H); 3.66 (s, 3H, CH_3O); 5.59–5.67 (m, 1H, H_3); 5.92–5.96 (br s, 1H, H_2); 5.96–6.04 (m, 1H, H_4); 6.06 (br s 1H, NH); 7.44–7.51 (m, 2H, Arom.); 7.57–7.63 (m, 1H, Arom.); 8.00–8.06 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 22.0, 23.2, 26.2 (C_5, C_6, CH_3CO); 52.6 (CH_3O); 60.0 (C_1); 70.8 (C_2); 123.3 (C_3); 128.4, 129.5, 132.2, 133.3 (Arom, C_4); 165.2, 170.4, 171.3 (COO, PhCOO, CON).

4.1.16. Methyl 1-acetamido-*t*-2-benzoyloxycyclohexane-*r*-1-carboxylate (21). A solution of compound **20** (350 mg, 1.10 mmol) in dry CH_2Cl_2 (10 mL) was hydrogenated at atmospheric pressure, using platinum–carbon (10%) as a catalyst (50 mg), vigorously stirring at 35°C for 14 h. The catalyst was filtered off through Celite and the solvent was evaporated. The residue was chromatographed on silica gel eluting with hexane–ethyl acetate (4:6), to yield 273 mg of compound **21** as a white solid (78%). Mp: 44–45°C; Anal. calcd for $C_{17}H_{21}NO_5$: C, 63.94; H, 6.63; N, 4.39; found: C, 63.37; H, 6.49; N, 4.31; ^{1}H NMR ($CDCl_3$): δ 1.40–1.62 (m, 3H); 1.71–1.85 (m, 2H); 2.05 (s, 3H, CH_3CO); 1.92–2.13 (m, 2H); 2.66–2.77 (m, 1H); 3.64 (s, 3H, CH_3O); 5.40 (dd, 1H, J_{2a-3a}=9.6 Hz, J_{2a-3e}=4.2 Hz, H_{2a}); 5.94 (br s, 1H, NH); 7.42–7.49 (m, 2H, Arom.); 7.55–7.62 (m, 1H, Arom.); 7.92–8.02 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 20.1, 22.3, 23.0, 27.0, 29.7 (C_3, C_4, C_5, C_6, CH_3CO); 52.2 (CH_3O); 62.0 (C_1); 73.2 (C_2); 128.2, 129.3, 129.5, 132.9 (Arom.); 164.9, 170.3, 171.5 (COO, PhCOO, CON).

4.1.17. 1-Amino-*t*-2-hydroxycyclohexane-*r*-1-carboxylic acid (*rac-cis*-1). Compound **21** (250 mg, 0.78 mmol) was suspended in a 6N HCl solution (20 mL) and stirred under reflux for 24 h. The solvent was evaporated and the excess of HCl was removed in vacuo. The residue was dissolved in distilled water (15 mL) and washed with Et_2O (2×20 mL). The aqueous layer was evaporated to yield 125 mg of *rac-cis*-**1**·HCl as a white solid (82%), which was dissolved in ethanol (6 mL) and propylene oxide (2 mL) was added. After stirring at reflux for 2 h, the amino acid precipitated partially. The solvent was evaporated and the residue was dissolved in distilled water (2 mL) and eluted through a C_{18} reverse-phase Sep-pak cartridge to yield, after removal of water, 95 mg of the amino acid *rac-cis*-**1** as a white solid (76%). *rac-cis*-**1**·HCl: ^{1}H NMR (D_2O): δ 1.15–1.40 (m,

3H); 1.50–1.80 (m, 2H); 1.81–1.90 (m, 3H); 4.13 (dd, 1H, J_{2a-3a}=10.8 Hz, $J_{2a-3e'}$=5.1 Hz, H_{2a}); ^{13}C NMR (D_2O): δ 20.9, 24.8, 30.6, 32.8 (C_3, C_4, C_5, C_6); 67.0 (C_1); 71.8 (C_2); 175.8 (COO). *rac-cis*-**1**: Anal. calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80; found: C, 52.72; H, 8.18; N, 8.92; ^{1}H NMR (D_2O): δ 1.10–1.50 (m, 3H); 1.55–1.80 (m, 2H); 1.82–2.00 (m, 3H); 4.02–4.09 (m, 1H, H_2); ^{13}C NMR (D_2O): δ 21.5, 25.2, 31.1, 33.3 (C_3, C_4, C_5, C_6); 68.1 (C_1); 72.1 (C_2); 178.3 (COO).

4.1.18. *trans*-2-Methoxycyclohexane-1-spiro-{4′ [2′-phenyl-5′(4′H)oxazolone]} (*rac*-22). A suspension of compound *rac*-**8** (500 mg, 1.72 mmol) and LiOH·H_2O (722 mg, 17.2 mmol) in methanol–water (3:2) (25 mL) was stirred under reflux for 7 h. The solvent was removed, the residue was then dissolved in water and washed with CH_2Cl_2 (2×20 mL). The aqueous phase was acidified with 2N HCl and then extracted with CH_2Cl_2 (4×25 mL). The combined last organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated, to yield 444 mg of the 1-benzamido-*c*-2-methoxycyclohexane-*r*-1-carboxylic acid as a white solid (93%). Mp: 49–51°C; Anal. calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05; found: C, 64.91; H, 6.93; N, 4.99; IR (CH_2Cl_2, cm^{-1}): 3430 (NH), 3119 (COOH), 1760 (COO), 1672 (CON); ^{1}H NMR ($CDCl_3$): δ 1.42–1.82 (m, 5H); 1.89–2.04 (m, 1H); 2.10–2.24 (m, 2H); 3.40 (s, 3H, CH_3O); 4.18–4.26 (m, 1H, H_2); 6.93 (br s, 1H, NH); 7.36–7.55 (m, 3H, Arom.); 7.74–7.85 (m, 2H, Arom.); 10.46 (br s, 1H, COOH); ^{13}C NMR ($CDCl_3$): δ 19.9, 20.9, 24.4, 28.6 (C_3, C_4, C_5, C_6); 57.1 (CH_3O); 62.5 (C_1); 77.7 (C_2); 127.1, 128.5, 131.9, 133.8 (Arom.); 167.9 (CON); 174.9 (COOH). This carboxylic acid (444 mg, 1.6 mmol) and dimethylaminopyridine (192 mg, 1.6 mmol) were dissolved in CH_2Cl_2 (13 mL) at 0°C. Dicyclohexylcarbodiimide (335 mg, 1.6 mmol) was then added and the mixture was allowed to warm up to 25°C. After stirring for 3 h, the solvent was evaporated and the residue was chromatographed on silica gel eluting with hexane–ethyl acetate (1:1), to yield 337 mg of the oxazolone *rac*-**22** as a white solid (91%). Mp: 82–84°C; Anal. calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40; found: C, 69.86; H, 6.43; N, 5.62; IR (CH_2Cl_2, cm^{-1}): 1805 (COO), 1657 (CON); ^{1}H NMR ($CDCl_3$): δ 1.29–1.45 (m, 1H); 1.58–1.72 (m, 1H); 1.76–2.02 (m, 5H); 2.03–2.14 (m, 1H); 3.31 (s, 3H, CH_3O); 3.49 (dd, 1H, J_{2a-3a}=11.2 Hz, J_{2a-3e}=4.9 Hz, H_2); 7.41–7.60 (m, 3H, Arom.); 7.95–8.06 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 19.8 (C_3), 23.6, 25.5, 33.0 (C_4, C_5, C_6); 58.0 (CH_3O); 73.0 (C_1); 82.3 (C_2); 126.0, 127.8, 128.7, 132.5 (Arom.); 160.8 (CON); 177.3 (COO).

4.1.19. (1*S*,2*S*)-[1-Benzamido-2-methoxycyclohexane-1-carboxamido]-(*S*)-phenylalanine cyclohexylamide ((1*S*,2*S*,*S*)-23) and (1*R*,2*R*)-[1-benzamido-2-methoxycyclohexane-1-carboxamido]-(*S*)-phenylalanine cyclohexylamide ((1*R*,2*R*,*S*)-24). A solution of (*S*)-phenylalanine cyclohexylamide (1.6 g, 6.66 mmol) in *N*-methylpyrrolidin-2-one (NMP) (3 mL) was added to a solution of compound *rac*-**22** (750 mg, 2.9 mmol) in NMP (3 mL) under an inert atmosphere. After stirring for 48 h at 90°C, the reaction mixture was allowed to cool down to room temperature and was then poured onto a mixture of ice (12 g), 1N HCl (12 mL) and ethyl acetate (15 mL). After stirring for 30 min, the organic phase was washed with water (2×15 mL) and the combined aqueous phases were extracted with ethyl acetate (2×15 mL). The combined organic phases were washed with brine (2×15 mL), dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The mixture of diastereoisomeric peptides was chromatographed on silica gel eluting with toluene–ethyl acetate (1:1), to yield 525 mg of the peptide (1*S*,2*S*,*S*)-**23** as a white solid (36%) and 514 mg of the peptide (1*R*,2*R*,*S*)-**24** as a white solid (35%). (1*S*,2*S*,*S*)-**23**: Mp: 192–194°C; Anal. calcd for $C_{30}H_{39}N_3O_4$: C, 71.26; H, 7.77; N, 8.31; found: C, 71.64; H, 7.62; N, 8.23; $[\alpha]^{25}_D$ (*c* 2.08, $CHCl_3$)=−32.1; IR (CH_2Cl_2, cm^{-1}): 3426, 3351 (NH), 1702, 1657 (CON); ^{1}H NMR ($CDCl_3$): δ 1.02–1.42 (m, 5H); 1.43–1.93 (m, 12H); 2.19–2.36 (m, 1H); 3.15 (s, 4H, CH_3O+$H_{\beta1}$); 3.27 (dd, 1H, $J_{\beta2-\beta1}$=14.1 Hz, $J_{\beta2-\alpha}$=6.9 Hz, $H_{\beta2}$); 3.64–3.82 (m, 1H, $H_{1'}$); 3.89–4.01 (m, 1H, H_2); 4.66–4.79 (m, 1H, H_α); 6.51 (br s, 1H, NHCOPh); 6.90 (d, 1H, $J_{NHCy-1'}$=8.7 Hz, NHCy); 7.10–7.18 (m, 5H, Arom.); 7.34–7.55 (m, 4H, Arom.+NHPhe); 7.63–7.72 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 20.0, 21.2, 22.9, 25.1, 25.2, 25.6, 30.7, 32.5, 32.8 (C_3, C_4, C_5, C_6, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$); 37.0 (C_β); 48.5 ($C_{1'}$); 53.9 (C_α); 55.8 (CH_3O); 61.8 (C_1); 78.2 (C_2); 126.6, 127.0, 128.4, 128.6, 129.4, 132.0, 133.8, 137.2 (Arom.); 167.4, 169.8, 172.3 (CON). (1*R*,2*R*,*S*)-**24**: Mp: 111–113°C; Anal. calcd for $C_{30}H_{39}N_3O_4$: C, 71.26; H, 7.77; N, 8.31; found: C, 71.89; H, 7.64; N, 8.42; $[\alpha]^{25}_D$ (*c* 1.02, $CHCl_3$)=−57.4; IR (CH_2Cl_2, cm^{-1}): 3427, 3348 (NH), 1805, 1658 (CON); ^{1}H NMR ($CDCl_3$): δ 0.92–2.02 (m, 17H); 2.09–2.22 (m, 1H); 3.08 (dd, 1H, $J_{\beta1-\beta2}$=14.1 Hz; $J_{\beta1-\alpha}$=6.0 Hz, $H_{\beta1}$); 3.21 (s, 3H, CH_3O); 3.26 (dd, 1H, $J_{\beta2-\beta1}$=14.1 Hz, $J_{\beta2-\alpha}$=6.6 Hz, $H_{\beta2}$); 3.64–3.79 (m, 1H, $H_{1'}$); 3.87–3.94 (m, 1H, H_2); 4.64–4.73 (m, 1H, H_α); 6.35 (br s, 1H, NHCOPh); 6.62 (d, 1H, $J_{NHCy-1'}$=7.8 Hz, NHCy); 7.00 (d, 1H, $J_{NHPhe-\alpha}$=7.8 Hz, NHPhe); 7.14–7.24 (m, 5H, Arom.); 7.39–7.48 (m, 2H, Arom.); 7.48–7.56 (m, 1H, Arom.); 7.68–7.77 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 19.9, 21.2, 24.2, 25.0, 25.5, 28.0, 32.7, 32.9, 33.9 (C_3, C_4, C_5, C_6, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$); 37.3 (C_β); 48.3 ($C_{1'}$); 54.0 (C_α); 58.8 (CH_3O); 62.1 (C_1); 78.6 (C_2); 126.7, 127.0, 128.4, 128.6, 129.4, 132.0, 133.9, 136.8 (Arom.); 167.2, 169.6, 172.0 (CON).

4.1.20. Methyl (1*S*,2*S*)-1-benzamido-2-methoxycyclohexane-1-carboxylate ((1*S*,2*S*)-8). Triflic acid (0.11 mL, 2.37 mmol) was added to a solution of (1*S*,2*S*,*S*)-**23** (400 mg, 0.79 mmol) in dry methanol (12 mL) at 0°C, under an inert atmosphere. The reaction mixture was warmed up to 80°C and after stirring for 48 h, the solvent was evaporated. The residue was chromatographed on silica gel eluting with hexane–ethyl acetate (1:1), to yield 210 mg of the compound (1*S*,2*S*)-**8** as a white solid (91%). Mp: 132–133°C; Anal. calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81; found: C, 65.84; H, 7.32; N, 4.75; $[\alpha]^{25}_D$ (*c* 1.09, $CHCl_3$)=+52.5. Spectral data for the compound (1*S*,2*S*)-8 were identical to those described for *rac*-**8**.

4.1.21. Methyl (1*R*,2*R*)-1-benzamido-2-methoxycyclohexane-1-carboxylate ((1*R*,2*R*)-8). As described for (1*S*,2*S*)-**8**, compound (1*R*,2*R*)-**8** (196 mg, 85%) was obtained starting from (1*R*,2*R*,*S*)-**24** (400 mg, 0.79 mmol). Anal. calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81; found: C, 65.80; H, 7.22; N, 4.79; $[\alpha]^{25}_D$ (*c* 1.00, $CHCl_3$)=−54.9. Analytical and spectral data for the

compound (1*R*,2*R*)-**8** were identical to those described for its enantiomer (1*S*,2*S*)-**8**.

4.1.22. (1*S*,2*S*)-1-Amino-2-hydroxycyclohexane-1-carboxylic acid ((1*S*,2*S*)-1). Compound (1*S*,2*S*)-**8** (220 mg, 0.76 mmol) was suspended in a 12N HCl solution (5 mL) and stirred under reflux for 7 d. The solvent was evaporated and the excess of HCl removed in vacuo. The residue was dissolved in distilled water (8 mL) and washed with Et_2O (4×10 mL). The aqueous layer was evaporated to yield 133 mg (90%) of (1*S*,2*S*)-**1**·HCl as a white solid, which was dissolved in ethanol (3 mL) and propylene oxide (1 mL) was added. After stirring under reflux for 2 h, the amino acid precipitated partially. The solvent was evaporated and the residue was dissolved in distilled water (2 mL) and eluted through a C_{18} reverse-phase Sep-pak cartridge to yield, after removal of water, 100 mg of the amino acid (1*S*,2*S*)-**1** as a white solid (83%). Anal. calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80; found: C, 52.70; H, 8.20; N, 8.89; $[\alpha]^{25}_D$ (*c* 0.62, H_2O)=+4.5. Analytical and spectral data for the amino acid and its hydrochloride were identical to those described for *rac-trans*-**1**.

4.1.23. (1*R*,2*R*)-1-Amino-2-hydroxycyclohexane-1-carboxylic acid ((1*R*,2*R*)-1). As described for (1*S*,2*S*)-**1**, compound (1*R*,2*R*)-**1** (75 mg, 82%) was obtained starting from (1*R*,2*R*)-**8** (170 mg, 0.58 mmol). Anal. calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80; found: C, 52.76; H, 8.21; N, 8.90; $[\alpha]^{25}_D$ (*c* 0.62, H_2O)=−4.2. Analytical and spectral data for the amino acid and its hydrochloride were identical to those described for *rac-trans*-**1**.

4.1.24. Methyl (1*S*,2*R*,*S*)-1-(2′-acetoxypropanoylamido)-2-benzoyloxy-3-cyclohexene-1-carboxylate ((1*S*,2*R*,*S*)-25) and methyl (1*R*,2*S*,*S*)-1-(2′-acetoxypropanoylamido)-2-benzoyloxy-3-cyclohexene-1-carboxylate ((1*R*,2*S*,*S*)-26). Compound **19** (500 mg, 1.28 mmol) was dissolved in dry CH_2Cl_2 (50 mL), at 25°C under an inert atmosphere, and then Et_3N (0.36 mL, 2.57 mmol) and (*S*)-2-acetoxypropanoyl chloride (0.32 mL, 2.57 mmol) were added. After stirring for 2 h at the same temperature, the reaction was washed with a 0.005N HCl solution (50 mL) and the aqueous phase extracted with CH_2Cl_2 (2×50 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The mixture of diastereoisomers was chromatographed on silica gel eluting with Et_2O–hexane (7:3) to yield 200 mg of the compound (1*S*,2*R*,*S*)-**25** (40%) and 252 mg of the compound (1*R*,2*S*,*S*)-**26** (50%) as colourless oils. (1*S*,2*R*,*S*)-**25**: Anal. calcd for $C_{20}H_{23}NO_7$: C, 61.69; H, 5.95; N, 3.60; found: C, 61.53; H, 5.99; N, 3.66; $[\alpha]^{25}_D$ (*c* 1.02, CH_3OH)=−138.8; IR (CH_2Cl_2, cm^{-1}): 3437 (NH), 1744, 1738 (COO), 1690 (CON); ^1H NMR ($CDCl_3$): δ 1.47 (d, 3H, *J*=6.9 Hz, C*H*$_3$CH); 2.04 (s, 3H, CH_3CO); 2.07–2.27 (m, 3H); 2.83–2.89 (m, 1H); 3.66 (s, 3H, CH_3O); 5.18 (c, 1H, *J*=6.9 Hz, CH_3CH); 5.60–5.64 (m, 1H, H_4); 5.94 (m, 1H, H_2); 6.02–6.06 (m, 1H, H_3); 6.68 (br s, 1H, NH); 7.43–7.48 (m, 2H, Arom.); 7.57–7.63 (m, 1H, Arom.); 7.99–8.02 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 17.8 (*C*H_3CH); 20.9 (*C*H_3CO); 22.0, 26.3 (C_5, C_6); 52.9 (CH_3O); 60.0 (C_1); 70.7 (CH_3CH); 71.5 (C_2); 123.1 (C_4); 128.6, 129.5 (Arom.); 132.7 (C_3); 133.6 (Arom.); 165.2, 169.4, 170.8, 171.0 (COO, CON). (1*R*,2*S*,*S*)-**26**: Anal. calcd for $C_{20}H_{23}NO_7$: C, 61.69; H, 5.95; N, 3.60; found: C, 61.61; H, 5.92; N, 3.71; $[\alpha]^{25}_D$ (*c* 0.87, CH_3OH)=+125.4; IR (CH_2Cl_2, cm^{-1}): 3341 (NH), 1744, 1730 (COO), 1692 (CON); ^1H NMR ($CDCl_3$): δ 1.45 (d, 3H, *J*=6.9 Hz, C*H*$_3$CH); 2.06 (s, 3H, CH_3CO); 2.08–2.26 (m, 3H); 2.81–2.88 (m, 1H); 3.67 (s, 3H, CH_3O); 5.21 (c, 1H, *J*=6.9 Hz, CH_3CH); 5.56–5.62 (m, 1H, H_4); 5.95–6.02 (m, 2H, H_2+H_3); 6.72 (br s, 1H, NH); 7.45–7.50 (m, 2H, Arom.); 7.58–7.63 (m, 1H, Arom.); 8.02–8.05 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 17.3 (*C*H_3CH); 20.9 (*C*H_3CO); 22.0, 26.3 (C_5, C_6); 52.9 (CH_3O); 60.1 (C_1); 70.8 (CH_3CH); 71.4 (C_2); 122.9 (C_4); 128.6, 129.6 (Arom.); 132.7 (C_3); 133.5 (Arom.); 165.1, 169.5, 170.8, 171.0 (COO, CON).

4.1.25. Methyl (1*S*,2*R*,*S*)-1-(2′-acetoxypropanoylamido)-2-benzoyloxycyclohexane-1-carboxylate ((1*S*,2*R*,*S*)-27). A solution of compound (1*S*,2*R*,*S*)-**25** (159 mg, 0.40 mmol) in deoxygenated ethyl acetate (15 mL) was hydrogenated at atmospheric pressure, using platinum–carbon (10%) as a catalyst (50 mg), vigorously stirring at 25°C for 3 h. The catalyst was filtered off through Celite and the solvent evaporated, to yield 158 mg of compound (1*S*,2*R*,*S*)-**27** as a colourless oil (99%). Anal. calcd for $C_{20}H_{25}NO_7$: C, 61.37; H, 6.44; N, 3.58; found: C, 61.46; H, 6.38; N, 3.61; $[\alpha]^{25}_D$ (*c* 1.25, CH_3OH)=−77.3; IR (CH_2Cl_2, cm^{-1}): 3436 (NH), 1744, 1727, 1694, 1604 (COO, CON); ^1H NMR ($CDCl_3$): δ 1.37–1.85 (m, 5H); 1.49 (d, 3H, *J*=6.9 Hz, C*H*$_3$CH); 1.92–2.20 (m, 2H); 2.06 (s, 3H, CH_3CO); 2.75–2.85 (m, 1H); 3.64 (s, 3H, CH_3O); 5.21 (c, 1H, *J*=6.9 Hz, CH_3CH); 5.38 (dd, 1H, J_{2-3a}=9.9 Hz, J_{2-3e}=4.5 Hz, H_2); 6.61 (br s, 1H, NH); 7.41–7.47 (m, 2H, Arom.); 7.56–7.60 (m, 1H, Arom.); 7.95–7.98 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 17.8 (*C*H_3CH); 20.3, 22.9, 27.5, 29.8 (C_3, C_4, C_5, C_6); 20.9 (CH_3CO); 52.8 (CH_3O); 62.7 (C_1); 71.0 (CH_3CH); 74.3 (C_2); 128.6, 129.5, 129.6, 133.5 (Arom.); 164.9, 169.3, 170.6, 171.3 (COO, CON).

4.1.26. Methyl (1*R*,2*S*,*S*)-1-(2′-acetoxypropanoylamido)-2-benzoyloxycyclohexane-1-carboxylate ((1*R*,2*S*,*S*)-28). As described for (1*S*,2*R*,*S*)-**27**, compound (1*R*,2*S*,*S*)-**28** (216 mg, 99%) was obtained starting from (1*R*,2*S*,*S*)-**26** (218 mg, 0.56 mmol). Anal. calcd for $C_{20}H_{25}NO_7$: C, 61.37; H, 6.44; N, 3.58; found: C, 61.42; H, 6.50; N, 3.52; $[\alpha]^{25}_D$ (*c* 0.94, CH_3OH)=+40.5; IR (CH_2Cl_2, cm^{-1}): 3433 (NH), 1744, 1730, 1695, 1605 (COO, CON); ^1H NMR ($CDCl_3$): δ 1.37–1.79 (m, 5H); 1.48 (d, 3H, *J*=6.9 Hz, C*H*$_3$CH); 1.97–2.17 (m, 2H); 2.07 (s, 3H, CH_3CO); 2.80–2.85 (m, 1H); 3.65 (s, 3H, CH_3O); 5.26 (c, 1H, *J*=6.9 Hz, CH_3CH); 5.40 (dd, 1H, J_{2-3a}=9.9 Hz, J_{2-3e}=4.2 Hz, H_2); 6.67 (br s, 1H, NH); 7.43–7.49 (m, 2H, Arom.); 7.57–7.61 (m, 1H, Arom.); 8.00–8.03 (m, 2H, Arom.); ^{13}C NMR ($CDCl_3$): δ 17.2 (*C*H_3CH); 20.2, 22.9, 27.5, 29.9 (C_3, C_4, C_5, C_6); 20.9 (CH_3CO); 52.8 (CH_3O); 62.9 (C_1); 70.8 (CH_3CH); 74.2 (C_2); 128.6, 129.5, 129.6, 133.4 (Arom.); 164.9, 169.6, 170.6, 171.3 (COO, CON).

4.1.27. (1*S*,2*R*)-1-Amino-2-hydroxycyclohexane-1-carboxylic acid ((1*S*,2*R*)-1). Compound (1*S*,2*R*,*S*)-**27** (108 mg, 0.27 mmol) was suspended in a 9N HCl solution (10 mL). After stirring at 85°C for 4 d, the solvent was evaporated and the excess of HCl was removed in vacuo. The residue was washed with a mixture of THF–Et_2O (2:3) (2×5 mL) to obtain another residue, which was dried in vacuo to yield

49 mg (93%) of (1*S*,2*R*)-**1**·HCl as a white solid. This hydrochloride was dissolved in ethanol (2 mL) and propylene oxide (1 mL) was added. After stirring under reflux for 1 h, the amino acid precipitated and, once filtered, it was lyophilised to obtain 33 mg of the amino acid (1*S*,2*R*)-**1** as a white solid (85%; 77% from (1*S*,2*R*,*S*)-**27**). Anal. calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80; found: C, 52.81; H, 8.20; N, 8.86; $[\alpha]^{25}_D$ (*c* 1.17, H_2O)=+5.8. Analytical and spectral data for the amino acid and its hydrochloride were identical to those described for *rac-cis*-**1**.

4.1.28. (1*R*,2*S*)-1-Amino-2-hydroxycyclohexane-1-carboxylic acid ((1*R*,2*S*)-1). As described for (1*S*,2*R*)-**1**, compound (1*R*,2*S*)-**1** (28 mg, 75%) was obtained starting from (1*R*,2*S*,*S*)-**28** (107 mg, 0.27 mmol). Anal. calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80; found: C, 52.76; H, 8.15; N, 8.92; $[\alpha]^{25}_D$ (*c* 0.84, H_2O)=−5.5. Analytical and spectral data for the amino acid and its hydrochloride were identical to those described for *rac-cis*-**1**.

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References

1. Walsh, C. *Enzyme Reaction Mechanisms*; Freeman: San Francisco, 1979.
2. Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1887–1890.
3. Tjalsma, H.; Stover, A. G.; Driks, A.; Venema, G.; Bron, S.; Van Dijl, J.-M. *J. Biol. Chem.* **2000**, *275*, 25102–25108.
4. Higashimoto, Y.; Saito, S.; Tong, X.; Hong, A.; Sakaguchi, K.; Appella, E.; Anderson, C. W. *J. Biol. Chem.* **2000**, *275*, 23199–23203.
5. Sharma, P.; Barchi Jr., J. J.; Huang, X.; Amin, N. D.; Jaffe, H.; Pant, H. C. *Biochemistry* **1998**, *37*, 4759–4766.
6. Christensen, H. N.; Handlogten, M. E. *Biochim. Biophys. Acta* **1977**, *469*, 216–220.
7. Horikawa, M.; Ohfune, Y. *J. Synth. Org. Chem. Jpn* **1997**, *55*, 982–993.
8. Horikawa, M.; Kan, T.; Nakajima, T.; Nanba, K.; Ohfune, Y.; Takada, I. *Chirality* **1997**, *9*, 459–462.
9. Horikawa, M.; Nakajima, T.; Ohfune, Y. *Synlett* **1998**, 609–610.
10. Horikawa, M.; Nakajima, T.; Ohfune, Y.; Shigeri, Y.; Yoshikawa, S.; Yumoto, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2027–2032.
11. Fondekar, K. P.; Volk, F.-J.; Frahm, A. W. *Tetrahedron: Asymmetry* **1999**, *10*, 727–735.
12. Avenoza, A.; Cativiela, C.; Díaz-de-Villegas, M. D.; Peregrina, J. M. *Tetrahedron* **1993**, *49*, 10987–10996.
13. Avenoza, A.; Cativiela, C.; París, M.; Peregrina, J. M. *J. Org. Chem.* **1994**, *59*, 7774–7778.
14. Avenoza, A.; Cativiela, C.; Busto, J. H.; Peregrina, J. M.; Rodríguez, F. *Tetrahedron* **1998**, *54*, 11659–11674.
15. Jiménez, A. I.; Cativiela, C.; Avenoza, A.; París, M.; Peregrina, J. M.; Aubry, A.; Marraud, M. *Tetrahedron Lett.* **1998**, *39*, 7841–7844.
16. Jiménez, A. I.; Cativiela, C.; Gómez-Catalán, J.; Pérez, J. J.; Aubry, A.; París, M.; Marraud, M. *J. Am. Chem. Soc.* **2000**, *122*, 5811–5821.
17. Avenoza, A.; Cativiela, C.; Fernández-Recio, M. A.; Peregrina, J. M. *Synlett* **1995**, 891–892.
18. Avenoza, A.; Cativiela, C.; Fernández-Recio, M. A.; Peregrina, J. M. *Tetrahedron: Asymmetry* **1996**, *7*, 721–728.
19. Avenoza, A.; Cativiela, C.; Fernández-Recio, M. A.; Peregrina, J. M. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3375–3379.
20. Nakazawa, T.; Suzuki, T.; Ishii, M. *Tetrahedron Lett.* **1997**, *38*, 8951–8954.
21. Avenoza, A.; Busto, J. H.; Cativiela, C.; Fernández-Recio, M. A.; Peregrina, J. M.; Rodríguez, F. *Tetrahedron* **2001**, *57*, 545–548.
22. Avenoza, A.; Busto, J. H.; Cativiela, C.; París, M.; Peregrina, J. M. *J. Heterocycl. Chem.* **1997**, *34*, 1099–1110.
23. Avenoza, A.; Busto, J. H.; Cativiela, C.; Peregrina, J. M.; Zurbano, M. M. The use of 4-hetaryliden- and 4-aryliden-5(4*H*)-oxazolones as dienophiles in the Diels–Alder reaction; Attanasi, O. A., Spinelli, D., Eds.; In *Targets in Heterocyclic Systems. Chemistry and Properties*; Italian Society of Chemistry: Roma, 1999; vol. 3.
24. Avenoza, A.; Busto, J. H.; Cativiela, C.; Peregrina, J. M. *Amino Acids* **2000**, *18*, 117–127.
25. Rylander, P. N. In *Hydrogenation Methods*; Academic: London, 1990; pp 157–183.
26. Ranade, V. S.; Prins, R. *Chem. Eur. J.* **2000**, *6*, 313–320.
27. Obrecht, D.; Spiegler, C.; Schönholzer, P.; Müller, K.; Heimgartner, H.; Stierli, F. *Helv. Chim. Acta* **1992**, *75*, 1666–1696.
28. Obrecht, D.; Bohdal, U.; Broger, C.; Bur, D.; Lehmann, C.; Ruffieux, R.; Schönholzer, P.; Spiegler, C.; Müller, K. *Helv. Chim. Acta* **1995**, *78*, 563–580.
29. Obrecht, D.; Spiegler, C.; Schönholzer, P.; Müller, K.; Lehmann, C.; Ruffieux, R. *Helv. Chim. Acta* **1995**, *78*, 1567–1587.